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PLASMA CORTICOSTERONE LEVELS IN TWO SPECIES OF *ZONOTRICHIA* SPARROWS UNDER CAPTIVE AND FREE-LIVING CONDITIONS

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ABSTRACT.—We compared the daily plasma corticosterone (B) profiles in captive and free-living White-throated Sparrows (*Zonotrichia albicollis*) and White-crowned Sparrows (*Z. leucophrys*) during their non-breeding period. Neither sparrow species exhibited significant diel rhythms in the levels of corticosterone in captivity or under natural conditions, although the variations were suggestive of a rhythm. In each species, secretory profiles differed significantly between captive and free-living birds with mean B levels being 2–3 times higher in captive birds than in free-living individuals, despite the fact that captives had been "acclimated" for 35 days prior to sampling. Furthermore, mean B levels were two to four times higher in White-throated Sparrows under captive and free-living conditions than in White-crowned Sparrows under the same conditions. Our results indicate the need to use caution when (1) extrapolating such data obtained from captive individuals to those under natural conditions and (2) extrapolating data regarding B from one species to another. *Received 28 July 1994, accepted 1 Dec. 1994.*

Environmental stimuli may affect the concentrations and secretory patterns of hormones, such as the "stress-related" corticosteroid hormones, which can dictate physiological changes in organisms. In birds, the principal corticosteroid hormone is corticosterone (hereafter "B") (Assenmacher 1973). Perception of stressful stimuli markedly enhances the rate of B secretion through the actions of adrenal cortical stimulating hormone (ACTH), resulting in increased levels of B in the blood (Siegel 1971, 1980). In birds, this cause-effect relationship has been shown to occur in response to a variety of stressors (for review see Harvey et al. 1984), including thirst and starvation (Freeman et al. 1980, Scott et al. 1982), pollution (Holmes and Gorsline 1980), weather and temperature (Brown and Nestor 1973, Wingfield 1988), capture, handling and immobilization (Edens and Siegel 1975; Wingfield et al. 1982; Wingfield et al. 1992) and social stress (Gross and Siegel 1973, Satterlee et al. 1982).

Much of the current data on avian B profiles has been obtained from studies involving domesticated species or from wild species maintained in captivity (see references above). Data obtained from such studies are presumed to be similar to those which would be obtained from birds in

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nature. Since confinement itself or exposure to humans may be a stressor which might elevate B concentrations (Suarez and Gallup 1982, Hemsworth and Barnett 1989), especially in wild birds, the validity of these extrapolations needs to be tested. Determination of basal B levels is important in order to evaluate properly stress induced increases of B as well as for interspecific comparisons. Our first objective was to determine the basal B profiles of two species of sparrows (genus *Zonotrichia*) under natural conditions and compare these levels to birds acclimatized to captivity.

Seasonal variations in the recurring daily secretory profile of B have been observed in at least two avian species, the White-throated Sparrow (*Z. albicollis*) (Dusseau and Meier 1971) and the Garden Warbler (*Sylvia borin*) (Schwabl et al. 1991). Based on Dusseau and Meier's (1971) findings, Meier and Fivizzani (1975) proposed that the daily variations in B concentration occurring in different seasons reflects the dynamics of neuroendocrine mechanisms which directly control various aspects of seasonality in migratory birds, including premigratory fattening, migratory readiness, and breeding condition. Meier's hypothesis is supported primarily by studies of White-throated Sparrows maintained under captive conditions. In a similar study of the closely related White-crowned Sparrow (*Z. leucophrys*), Vleck et al. (1980) did not detect a recurring daily variation of B concentration. Thus, the second objective of our study was to compare the daily secretory profiles of B of these two congeners.

METHODS

White-throated Sparrows were mist-netted near Baton Rouge, Louisiana, on 24 and 25 January 1987 and placed in an outdoor aviary at Louisiana State University, Baton Rouge. White-crowned Sparrows were mist-netted near Charlotte, Texas, between 31 January and 2 February 1987, transported to Baton Rouge on 2 February, and placed in separate sections of the aviary housing the White-throated Sparrows. Both species were residing on their wintering grounds when captured. The outdoor aviaries in which the captive sparrows were maintained measured 6 × 4 × 4 m and were exposed to the natural local climate and photoperiod. At no time were birds exposed to any direct artificial lighting. Each enclosure held approximately 15 birds and contained numerous perches and abundant cover. A total of approximately 60 birds of each species were held in captivity for these experiments. During the acclimation period the aviary was entered only briefly once every three days to replenish food and water supplies. Water and a commercial bird seed (millet, sunflower seeds, corn mash) were provided ad libitum on the floor of the cage.

All birds were maintained under these conditions for approximately 35 days prior to having blood drawn for B assay (30–35 days is often used as an acclimation period prior to sampling captive wild birds). Blood samples were collected from captive White-throated Sparrows between 4 and 6 March and captive White-crowned Sparrows between 10 and 16 March. On each day, blood samples were collected from birds during six 1-h time periods (02:00–03:00, 06:00–07:00, 10:00–11:00, 14:00–15:00, 18:00–19:00, and 22:00–23:00). Individual birds were selected for blood collection by allowing a few individuals from a larger

group of birds to disperse passively to unoccupied sections of the aviary where they were netted. Generally, only two birds were sampled during each of the one-hour time periods per day, and sampling was done in more than one of the periods per day. This procedure permitted us to cause only minimal disturbance to the main group of birds and to the target bird prior to sampling blood. No bird was sampled more than once, and only those blood samples which were obtained within 60 sec of our initial effort to net a bird were assayed for B. Using only samples withdrawn during the initial 60 sec ensures that blood B levels do not reflect the stress of capture and handling associated with the blood sampling procedure (Wingfield et al. 1982, Schwabl et al. 1991). Blood was drawn by heart puncture, placed in heparinized tubes and kept on ice until plasma isolation.

For both species, blood samples were obtained from free-living birds at the same location where captive birds had been previously captured. Samples were collected during four one-hour time periods, beginning at first light and ending at dark (06:00–07:00, 10:00–11:00, 14:00–15:00, 18:00–19:00). Free-living White-throated Sparrows were collected between 25 February and 4 March and free-living White-crowned Sparrows between 7 and 9 March 1987. Individuals were collected from wintering flocks using .410 or .22 gauge bird shot. All possible efforts were taken to minimize disturbing birds while approaching flocks prior to collection and only one bird was collected in a particular flock per day to insure no residual stress from our prior disturbance. Blood samples were obtained by heart puncture within 60 sec of downing a bird, placed in heparinized tubes, and kept on ice until isolating and freezing plasma (within 2 h of collection). Birds were sexed by inspection of gonads and were deposited as specimens in the Louisiana State Univ. Museum of Natural Science.

Plasma was isolated from whole blood by centrifugation (10,000 × g for 60 sec), decanted and frozen at −20°C until assayed for B. The B concentration of each sample was determined by a single batch radioimmunoassay (Satterlee et al. 1980). For assay protocol, sensitivity, extraction efficiency, and specific-binding properties see Satterlee et al. (1980). Statistical comparisons were made by ANOVA or Student's *t*-test.

RESULTS

The concentration of B ranged from 4.2 ± 1.3 ng/ml (mean ± SE) (06:00 h, N = 6) to 9.5 ± 2.4 ng/ml (18:00 h, N = 7) in free-living White-throated Sparrows and from 13.8 ± 5.3 ng/ml (06:00 h, N = 6) to 31.3 ± 8.4 ng/ml (02:00 h, N = 6) in captive White-throated Sparrows (Fig. 1). In White-crowned Sparrows the plasma B concentrations ranged from 0.6 ± 0.2 ng/ml (14:00 h, N = 6) to 1.6 ± 1.0 ng/ml (06:00 h, N = 7) in free-living birds and 3.1 ± 1.1 ng/ml (18:00 h, N = 6) to 6.3 ± 2.5 ng/ml (06:00 h, N = 6) in captives (Fig. 1b). Despite variations, there were no significant differences in B levels among time periods for either captive ($F_{5,30} = 1.039$, $P < 0.50$) or free ($F_{3,22} = 1.1044$, $P > 0.50$)

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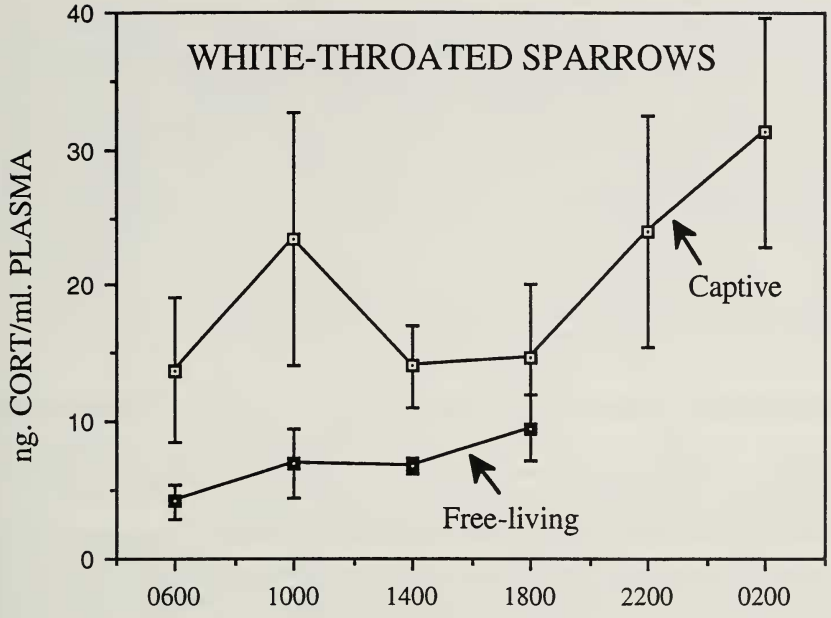
FIG. 1. Corticosterone (B) concentration (means and SE) by time of day for free-living and captive White-throated Sparrows and White-crowned Sparrows. Each point represents a sample of six individuals (except 10:00 and 18:00 in captive White-throated Sparrows, N = 7; and 06:00 and 10:00 in captive White-crowned Sparrows, N = 7). Note different scales on the y-axis.


WHITE-THROATED SPARROWS
ng. CORT/ml. PLASMA
40
30
20
10
0
Captive
Free-living
0600
1000
1400
1800
2200
0200

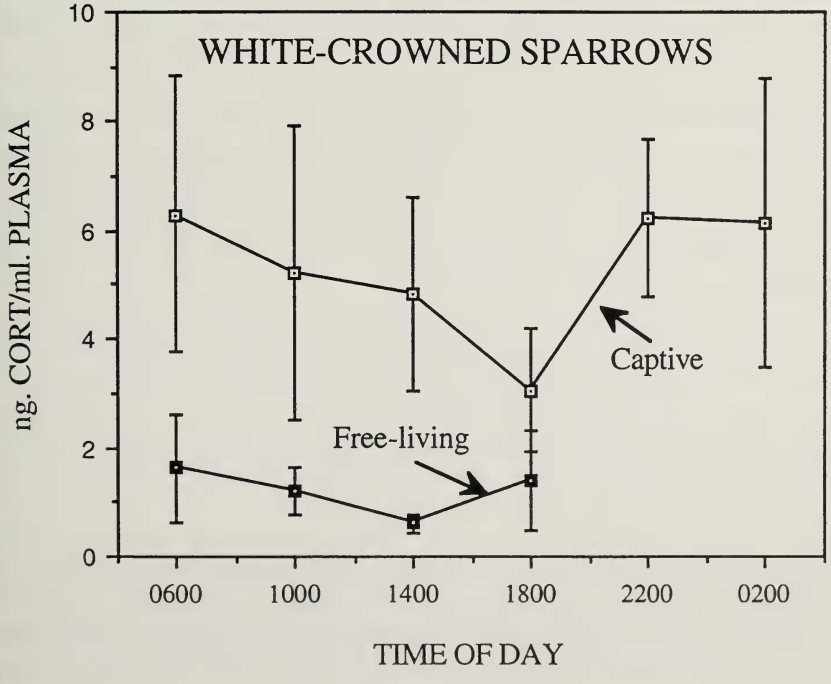

WHITE-CROWNED SPARROWS
ng. CORT/ml. PLASMA
10
8
6
4
2
0
Captive
Free-living
0600
1000
1400
1800
2200
0200
TIME OF DAY

TABLE 1

DAILY MEAN CORTICOSTERONE (NG/ML) FOR CAPTIVE AND FREE-LIVING WHITE-THROATED AND WHITE-CROWNED SPARROWS (N = SAMPLE SIZE)

Species[a]	Free-living mean ± SE	Captive mean ± SE
White-throated Sparrow	6.68 ± 1.0 N = 26	16.45 ± 2.9 N = 24
White-crowned Sparrow	1.16 ± 0.3 N = 26	4.93 ± 1.0 N = 24

[a] Species differed significantly under both treatments both between and within species, $P < 0.001$, *t*-test.

White-throated Sparrows, or captive ($F_{5,29} = 0.336$, $P > 0.75$) or free ($F_{3,23} = 0.5275$, $P < 0.75$) White-crowned Sparrows when tested by ANOVA. Furthermore there were no significant differences between B concentrations of males and females of either species.

Mean plasma B concentrations differed significantly between captive and free-living birds of the same species at most sampling times (Fig. 1) (*t*-test; $P < 0.05$). Corresponding values differed between captive and free-living birds, on average, by 260% for White-throated Sparrows and by 460% for White-crowned Sparrows (Fig. 1). A single daily-mean plasma B concentration was calculated for each experimental group by taking the mean of all values obtained between 06:00 and 18:00. B values from the 22:00–23:00 and 02:00–03:00 sample periods were not included in the daily-mean B concentration for captive birds since these comparable values were not available for free-living birds. The daily-mean B concentration was 250% and 430% greater in captive White-throated and White-crowned Sparrows than in their respective free-living counterparts (Table 1) (White-throated Sparrows, T = 3.20, $P < 0.003$; White-crowned Sparrows, T = 3.75, $P < 0.0008$).

Interspecific differences in daily-mean B concentration between these closely related congeners were also significant (Table 1). The daily-mean B concentration in free-living White-throated Sparrows was 580% greater than in free-living White-crowned Sparrows (T = 3.64, $P < 0.0009$), and 330% greater in captive White-throated Sparrows than in captive White-crowned Sparrows (T = 5.37, $P < 0.00002$).

DISCUSSION

Recurring daily variations in plasma B concentration have been detected in four domesticated species: Turkey (*Meleagris gallopavo*; Davis and Siopes 1988), Japanese Quail (*Coturnix coturnix*; Boissin and Assenmacher 1970, Assenmacher and Boissin 1972), Rock Dove (*Columba*

livia; Joseph and Meier 1973), and Domestic Fowl (*Gallus domesticus*; Webb and Mashaly 1985, Lauber et al. 1987). Similar variation was detected in two wild species maintained in captivity, White-throated Sparrow (Dusseau and Meier 1971, Meier and Fivizzani 1975) and Garden Warbler (Schwabl et al. 1991), but was not present in another wild species, the White-crowned Sparrow (Vleck et al. 1980). The occurrence of daily variations in plasma B levels in some species poses a problem for investigators if the levels of B are not consistently assessed at the same phase of the daily secretory profile. Furthermore, the fact that a daily variation of B concentration occurs in some species and is absent in others raises questions regarding the physiological significance of such hormone cycles. The period of the annual cycle also needs to be considered. We examined B levels at only one stage of the annual cycle which could very well explain our lack of significance.

Total plasma B concentration, as measured in the present study, includes B which is protein-bound and the bioactive fraction which is not protein-bound. It is possible that the plasma concentration of bioactive B could vary somewhat independently of the total plasma B concentration. However, Meier et al. (1978) examined this possibility in captive White-throated Sparrows and found that both total and bound plasma B varied similarly during the day. A close correlation between changes in plasma B level and relative concentration of protein-bound B has been observed in other species as well (Siegel et al. 1976, Kovács and Péczely 1983).

Significant daily variations of plasma B concentrations were not detected in captive sparrows of either species in this study. This observation is in accord with the findings of Vleck et al. (1980) for captive White-crowned Sparrows. However, our data are suggestive of a daily variation in B for both species.

At present, the cause of the differences of B concentration between captive and free-living birds of both species is unknown. To minimize external factors that might contribute to differences in B levels, we examined B concentrations in each experimental group of birds at the same times of day, and at the same time of year. Furthermore, free-living and captive birds of each species were sampled from the same locations.

Despite these precautions, we found highly significant differences in plasma B concentrations between captive and free-living birds for both species. This overall difference between captive and free-living birds is most likely due to increased ACTH release, possible due to a decreased level of B-feedback on corticotropin releasing factor (CRF) or increased adrenal sensitivity to ACTH, perhaps due to captive stress. Sustained differences in adrenal sensitivity to ACTH have been demonstrated in populations of chickens (Edens and Siegel 1975, Siegel 1973).

The differences in daily-mean B concentration between captive and free-living birds of the same species were especially surprising, given the length of the acclimation period. Wingfield et al. (1982) demonstrated a decrease in B levels after an acclimation period of two to three weeks for White-crowned Sparrows kept in small cages with one, two, or three individuals per cage. Our data suggest that our captive birds never fully "acclimated." In addition to the artificial stress imposed, there may have been additional social stressors of being in a confined space with other individuals. It is also possible that there was a residual stress effect from prior disturbances in the aviary, although we tried to minimize these as much as possible. Also, despite efforts to cause minimal disturbance, we may have induced some stress response during the sampling periods. One other possible explanation for the high B concentrations in "acclimated" birds may be change in diet. However, we don't believe that this was the case since seeds are the primary food for these sparrows in winter. There is no evidence to suggest that B concentrations are altered after subtle changes in diet. Furthermore, we did not observe any overt behavioral or physiological adversity in either species during the study period.

Although B concentrations were significantly different between captive and free-living birds, it is difficult to assess the biological significance of these differences in magnitude. Studies investigating the response of B to natural stressors in the wild and the potential impact that these resulting elevated B levels have on general fitness are long overdue.

The significant difference in B levels between these two congeners was unexpected. Both species are comparable in size, occupy similar habitats, and seem to exhibit similar behaviors while on the wintering grounds (P. P. Marra, pers. obs.). Furthermore, all birds were sampled at the same time of year at similar latitudes, and sample populations in both cases consisted of nearly equal numbers of male and female birds. Also, although we detected a species difference in the magnitude of increase in B levels owing to captivity, it seems unlikely that the difference found between free-living White-throated and White-crowned sparrows is "stress-related," unless these two species are continually subject to different degrees of stress in the wild. It is possible that the interspecific differences in B concentration reflect differences between the metabolic states of these species, perhaps caused by differences in habitat use, food availability, or reproductive readiness.

Given the dissimilarities among the daily profiles of B in the four experimental groups of birds examined, it seems unlikely that a particular secretory profile of plasma B serves a central role in determining the seasonal condition of these birds, at least at this phase of the annual cycle. This does not mean that a diel rhythm of B and other neurotransmitters

is not involved in establishing seasonality in birds. It is possible that a diel rhythm of plasma B is prominent at other phases of the annual cycle. Seasonal variations in plasma B levels have indeed been observed in several species (Meier and Fivizzani 1975, Wingfield et al. 1982, Hissa et al. 1983, Meier and Russo 1984, Wingfield 1985, Rehder et al. 1986).

To our knowledge, this is the first study in which daily profiles of plasma B have been compared between congeners, directly between birds in the wild and those maintained in captivity. Our results indicate significant differences not only between captive and free-living individuals within a species but also between congeners. Therefore, we suggest that extrapolations concerning B data be made with extreme caution. Furthermore, despite the lack of a significant daily variation of concentration, plasma B concentrations should be assessed, either at the same time or during predetermined times of day.

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